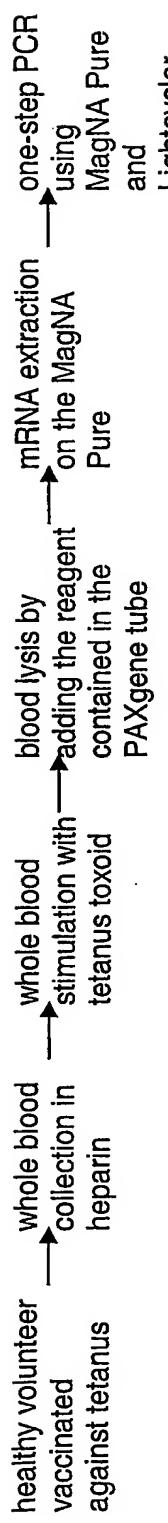


**Figures****Figure 1. Strategies followed in the given examples****Figure 1.1 *Ex vivo* monitoring of immune response against tetanus toxoid.**

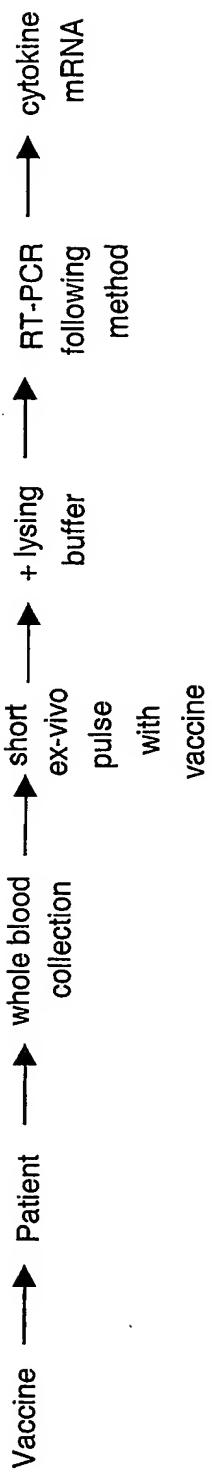
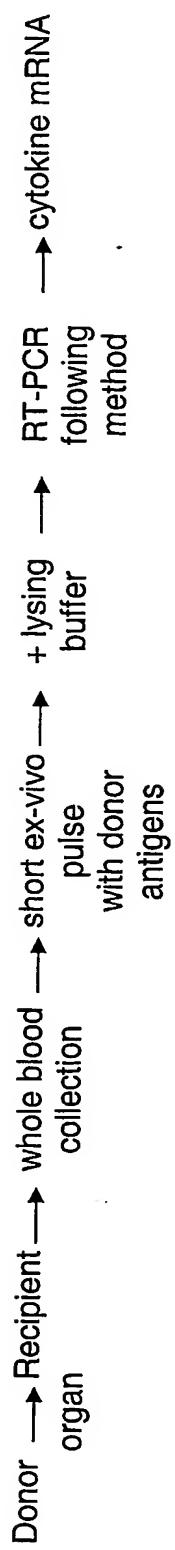


Figure 1.2. Strategy followed in example 3.



Figure 1.3 Strategy followed in example 4

Figure 1.4 Strategy followed in example 5.



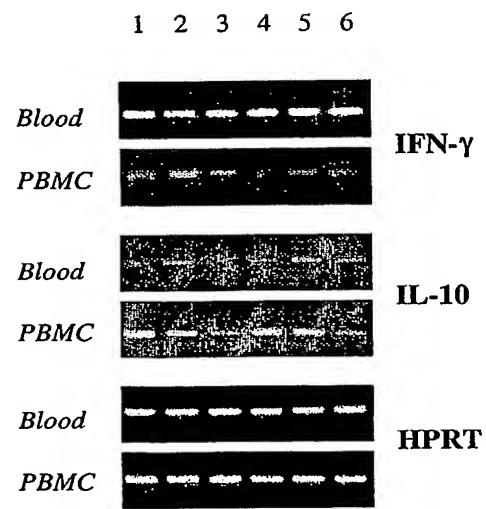


Figure 2.1

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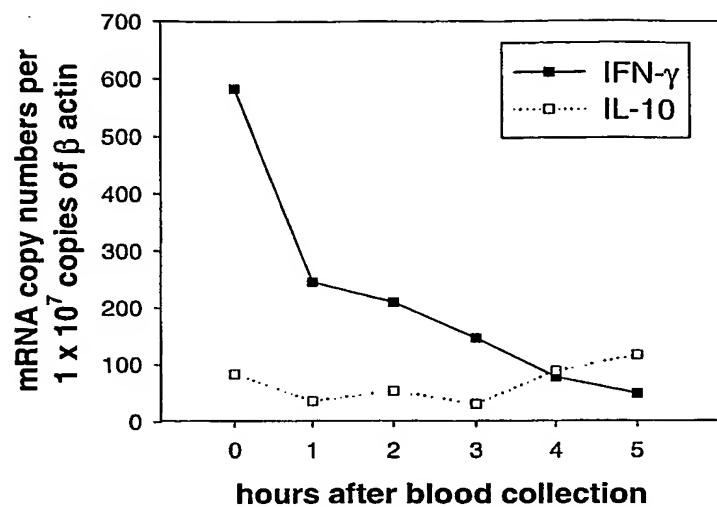
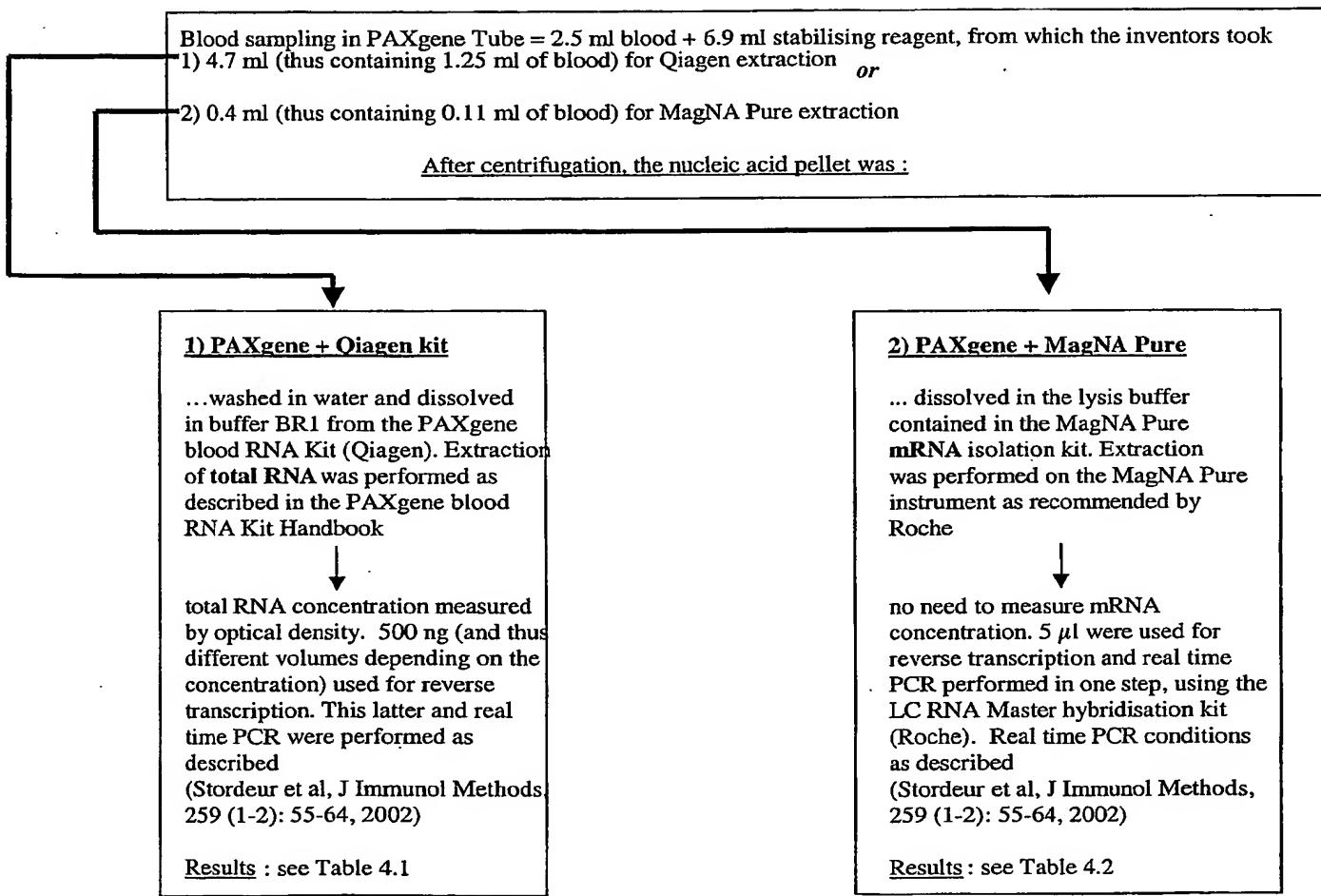


Figure 2.2

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Figure 3:



RECOMMENDED PROCEDURE

PROPOSED PROCEDURE

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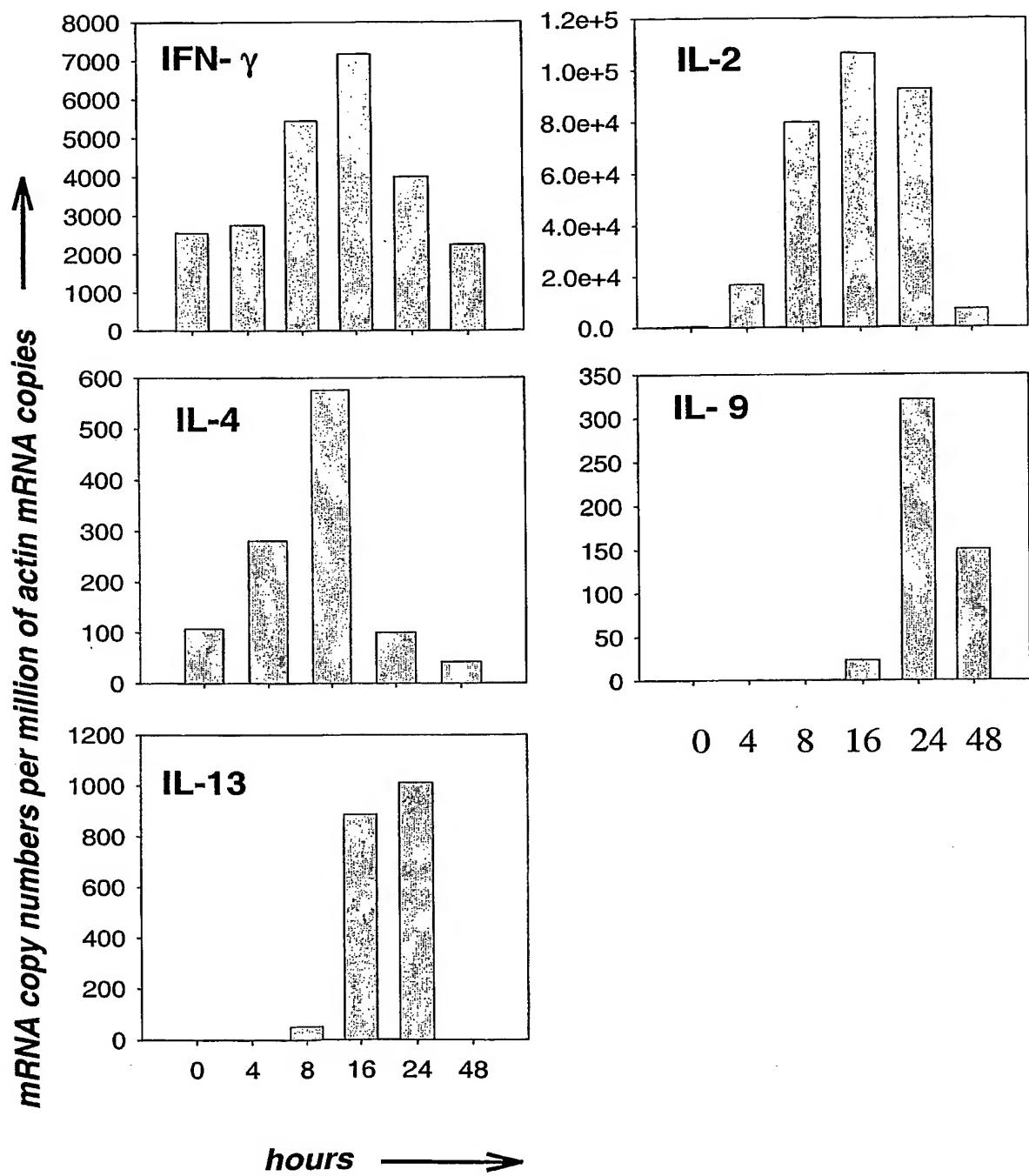


Figure 4

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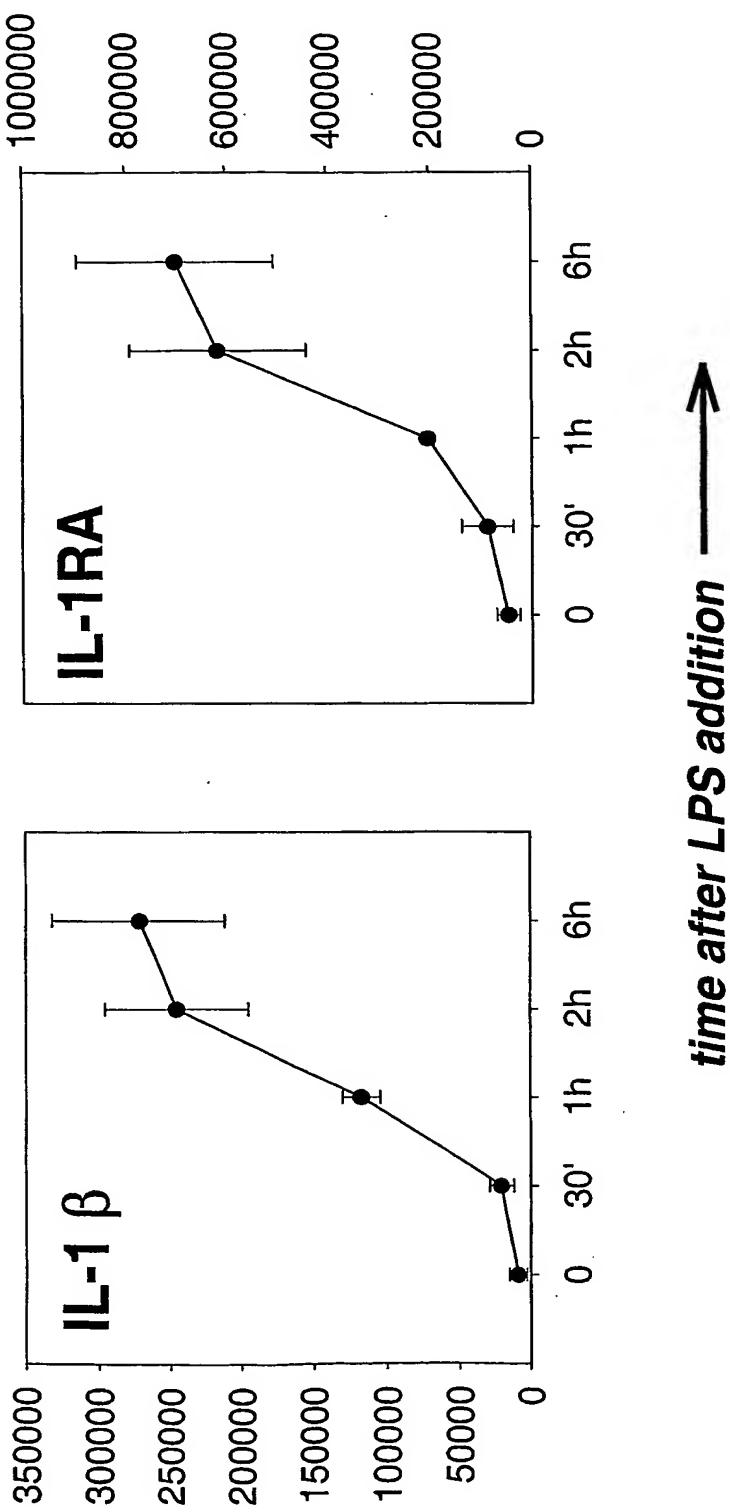


Figure 5

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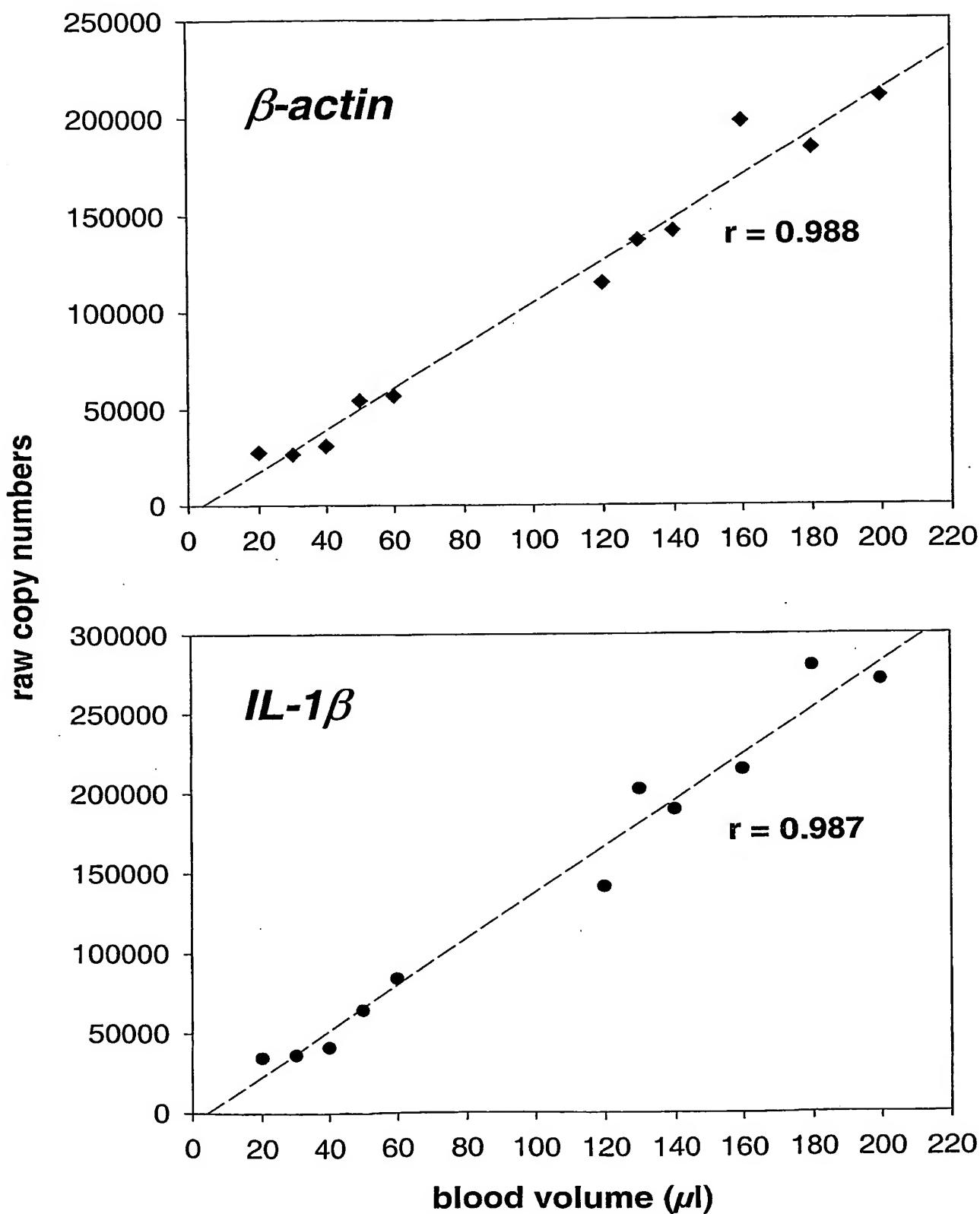


Figure 6

Figure 7

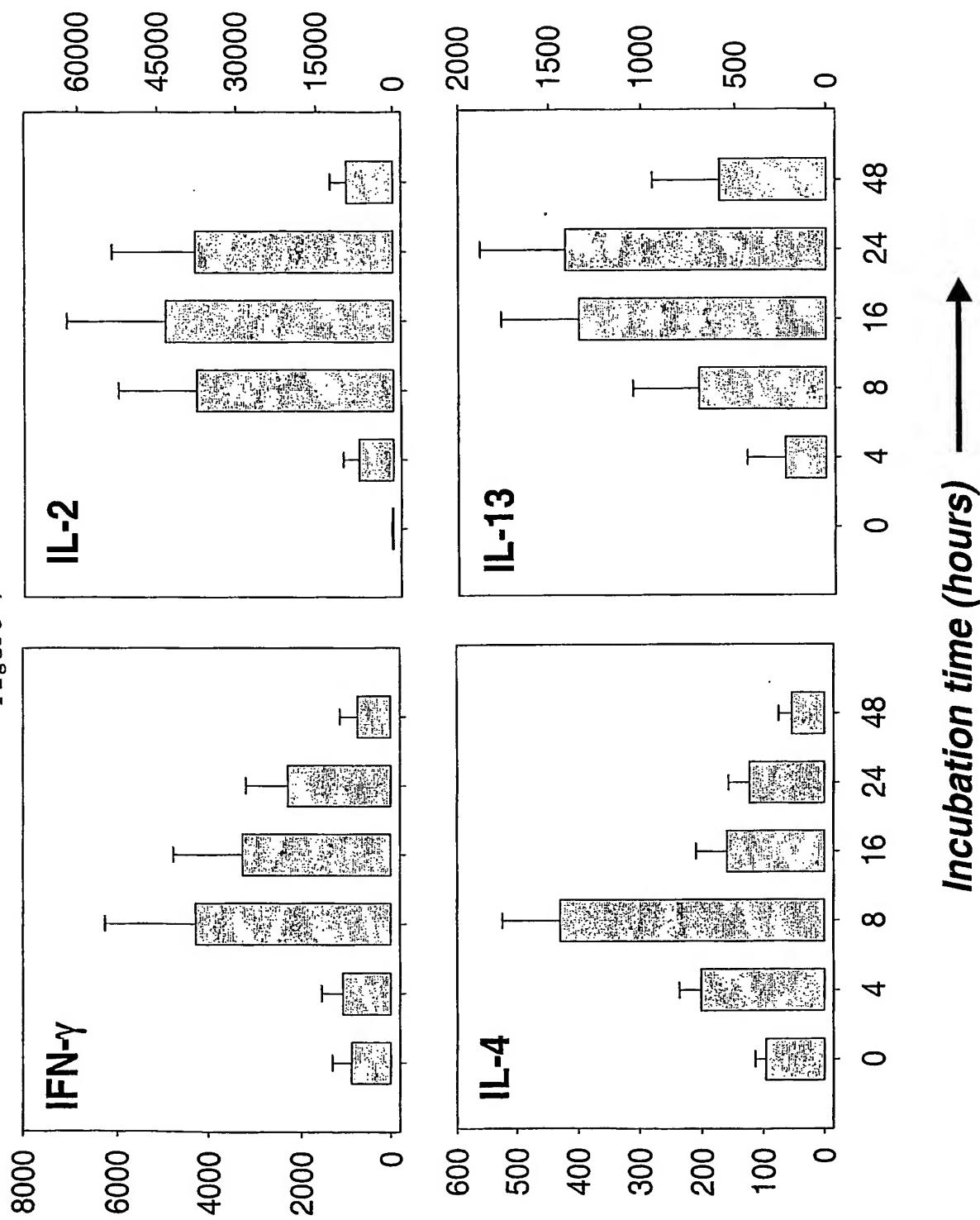


Figure 8

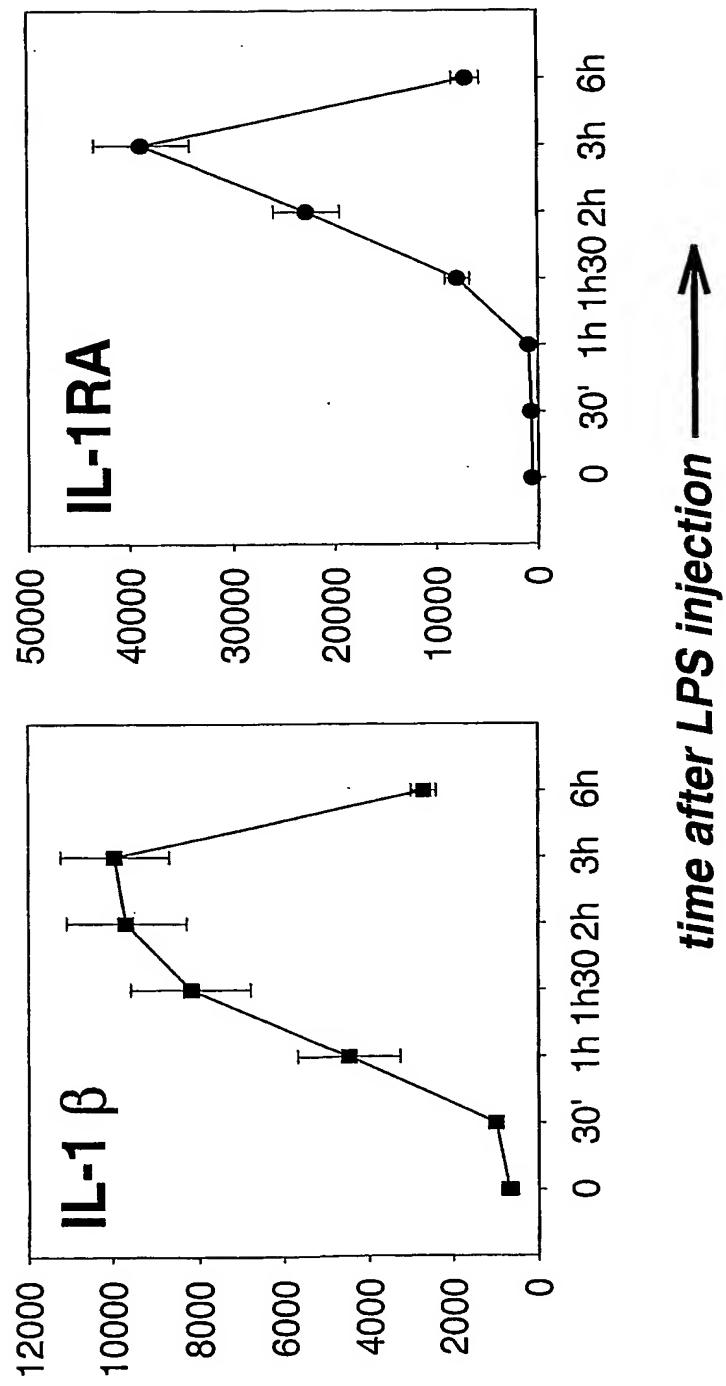
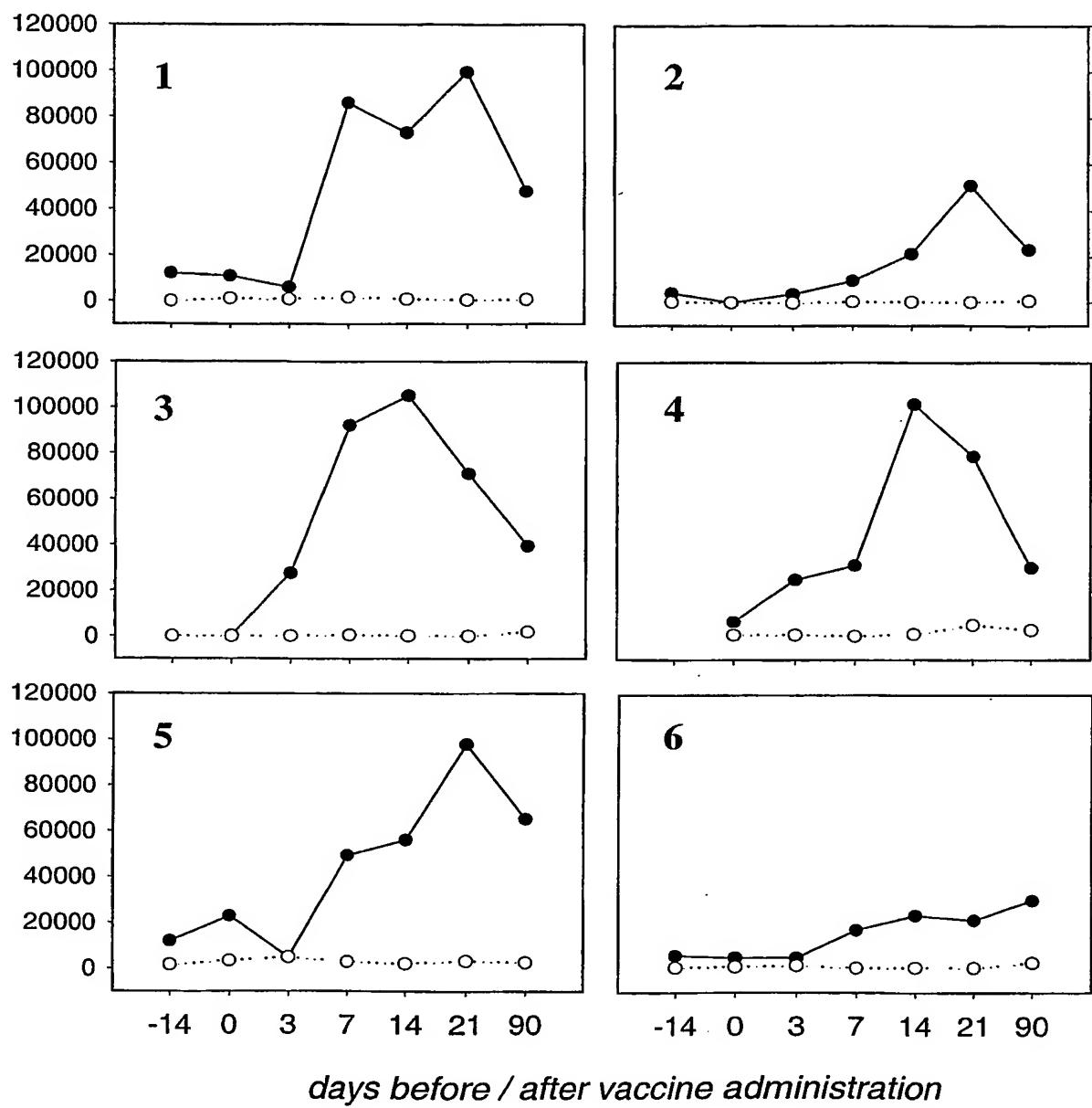


Figure 9



# Procedure to analyse blood cytokine mRNA expression

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- Whole blood sample incubation in the presence the tested potential immuno-modulator (antigen, allergen, cell, xenobiotic,...)
- Blood cells lysis and mRNA stabilisation using the reagent contained in the *PAXgene* tube
- Automated mRNA extraction and preparation of the reaction mixture for one-step RT-PCR using an automated device (*Magna Pure instrument – Roche Diagnostics*)
- Quantification of cytokine mRNA levels by **real-time PCR** using the *Lightcycler instrument (Roche Diagnostics)*

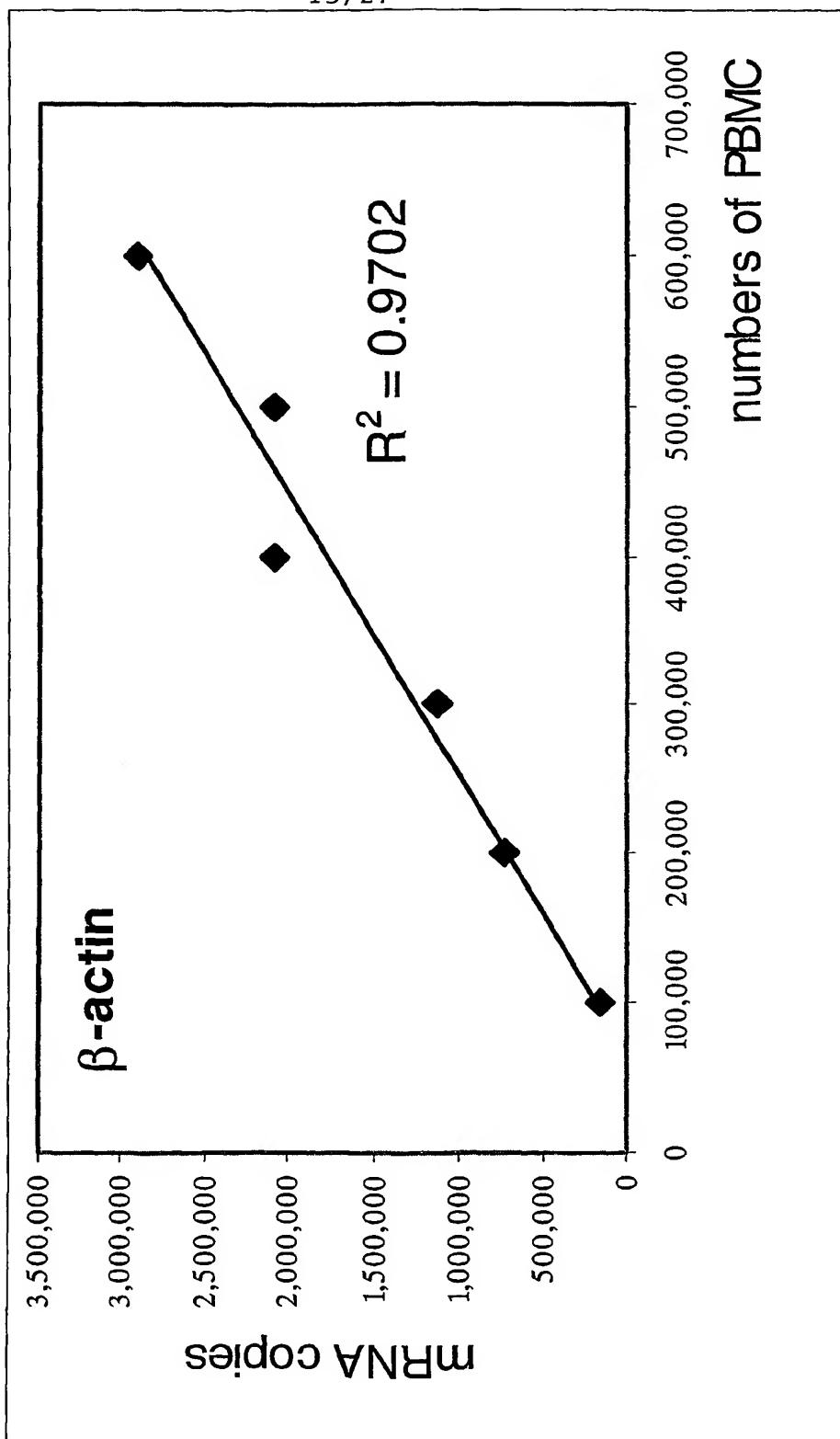
Figure 10

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# Automated mRNA extraction and reagent mix preparation on the MagNA Pure : direct correlation between starting cell number and found copy number

Figure 11



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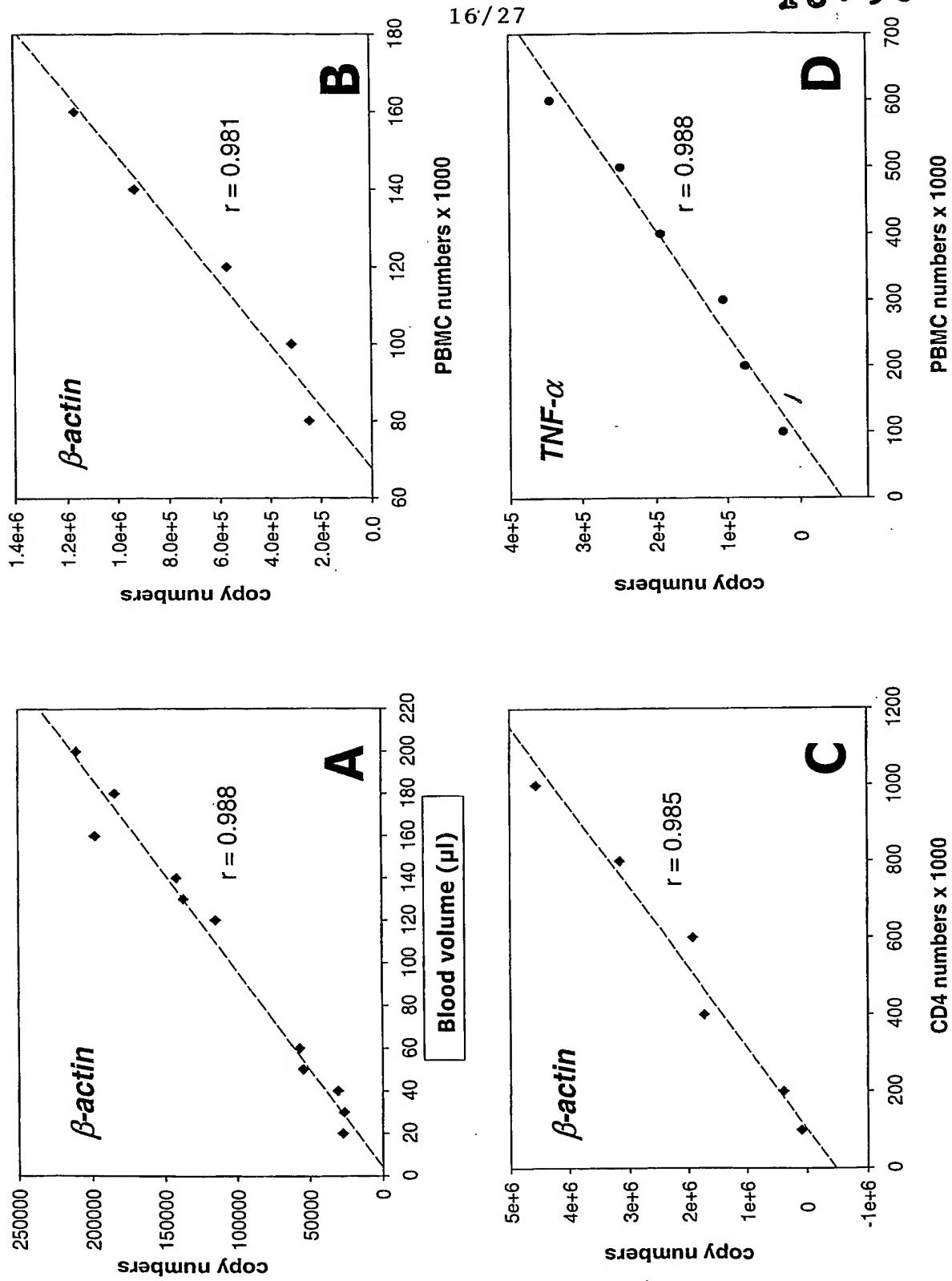


Figure 12

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# Immune monitoring in cancer immunotherapy

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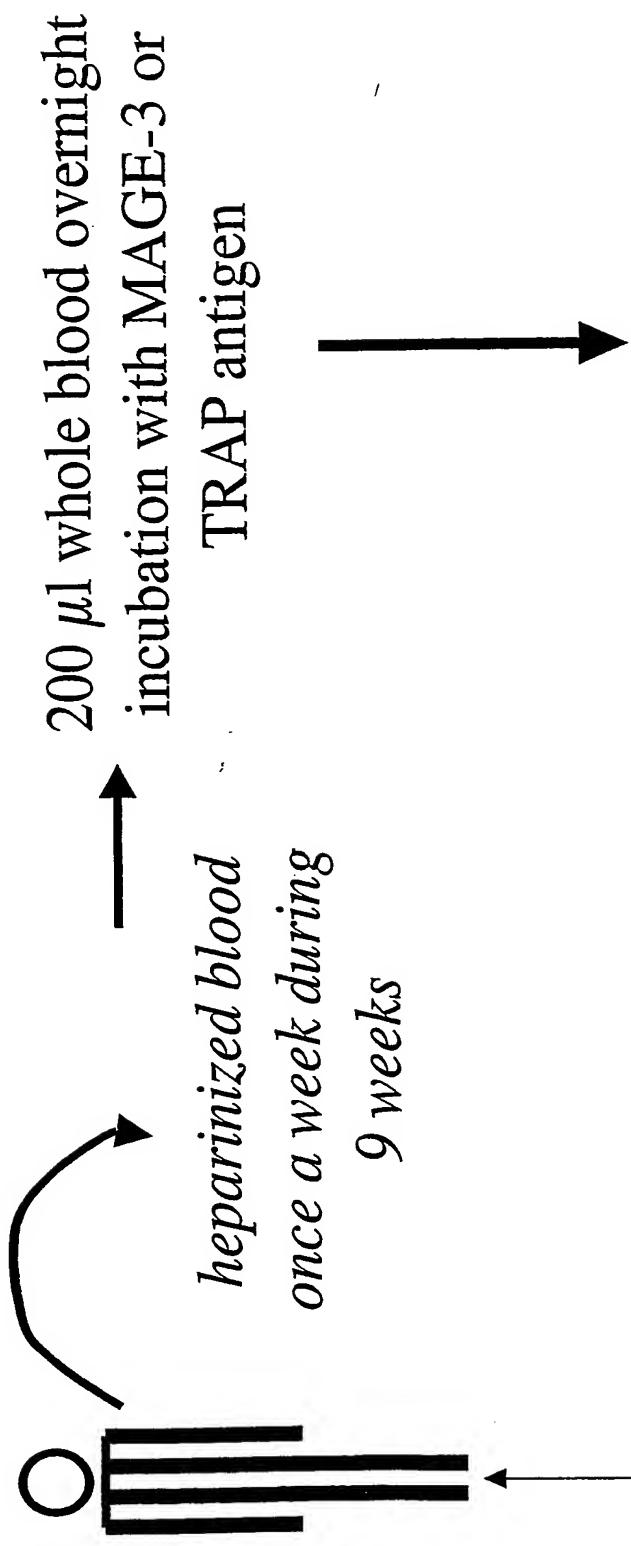
## Patient #3

- 7/99: melanoma of the right scapula. Excision
- 8/01: SC M+ arm, back, abdomen, right testis
- 4/02: right orchidectomy
- 4/02: pre-vaccine check-up
- 5/02: initiation of the vaccine program

Vaccine: MAGE-3 recombinant protein + adjuvant

Figure 13

# *In vitro stimulation of whole blood to assess immune response to MAGE-3*



Patient #3 received 3 injections of anti-MAGE-3 vaccine (MAGE-3 + adjuvant)

mRNA extraction and RT-PCR using the Magna Pure and the Lightcycler

Figure 14

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# IL-2 mRNA in whole blood following MAGE-3 vaccination in Patient #3

## IL-2 mRNA

(copy numbers per million of  $\beta$ -actin mRNA copies)

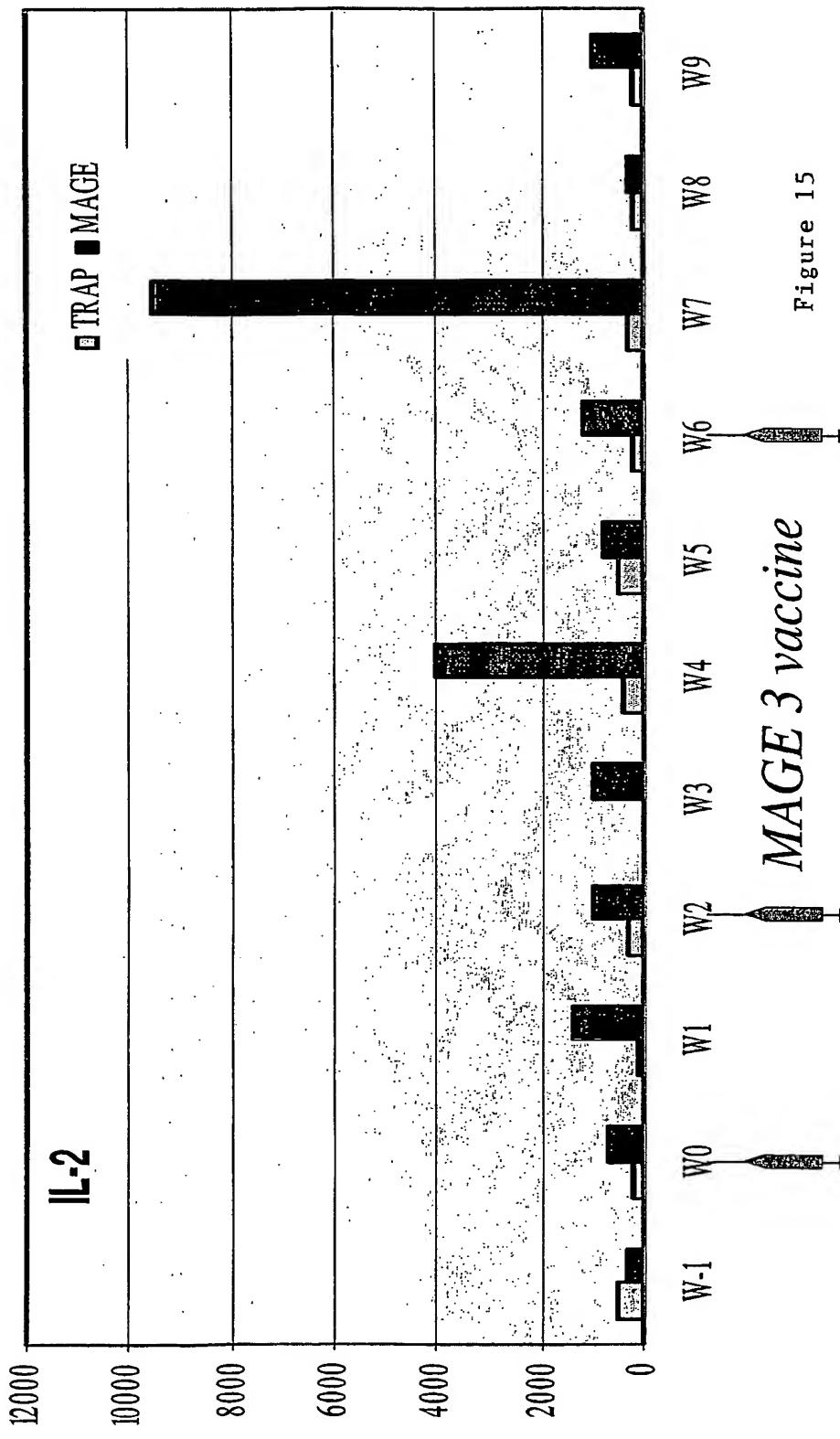
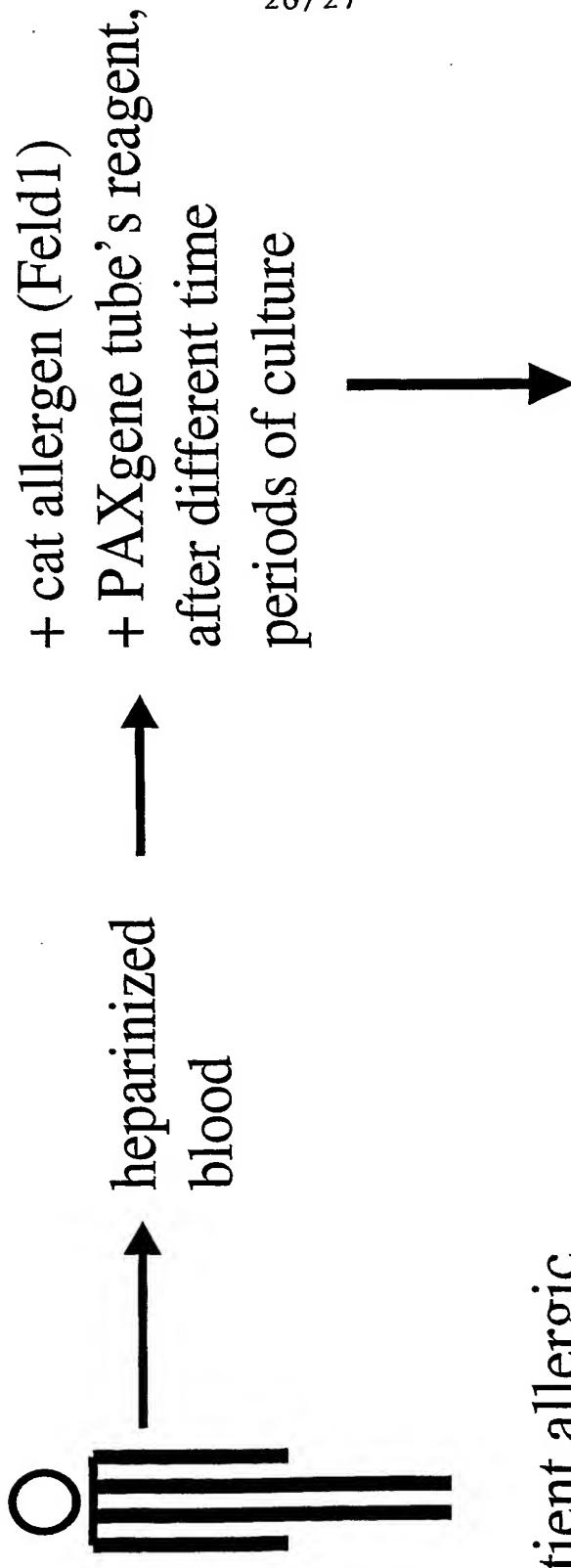


Figure 15

# *In vitro stimulation of whole blood : evaluation of immune response to allergen*



patient allergic  
to the cat or  
non-allergic controls

mRNA extraction and RT-PCR  
using the MagNA Pure and the  
Lightcycler

Figure 16

*In vitro stimulation of whole blood :  
evaluation of immune response to Feld1*

IL-4 mRNA copy numbers per million of  $\beta$  actin mRNA

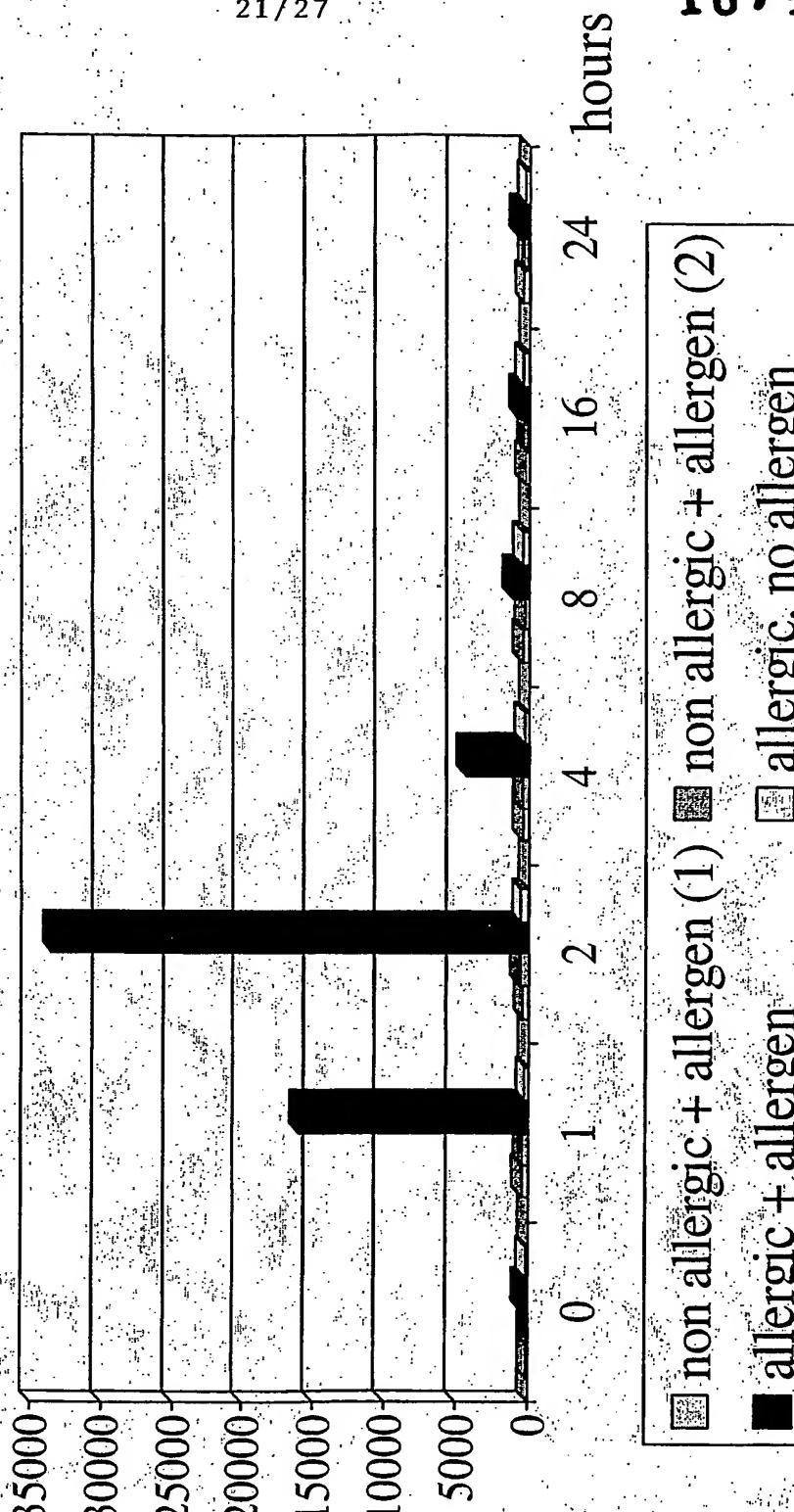
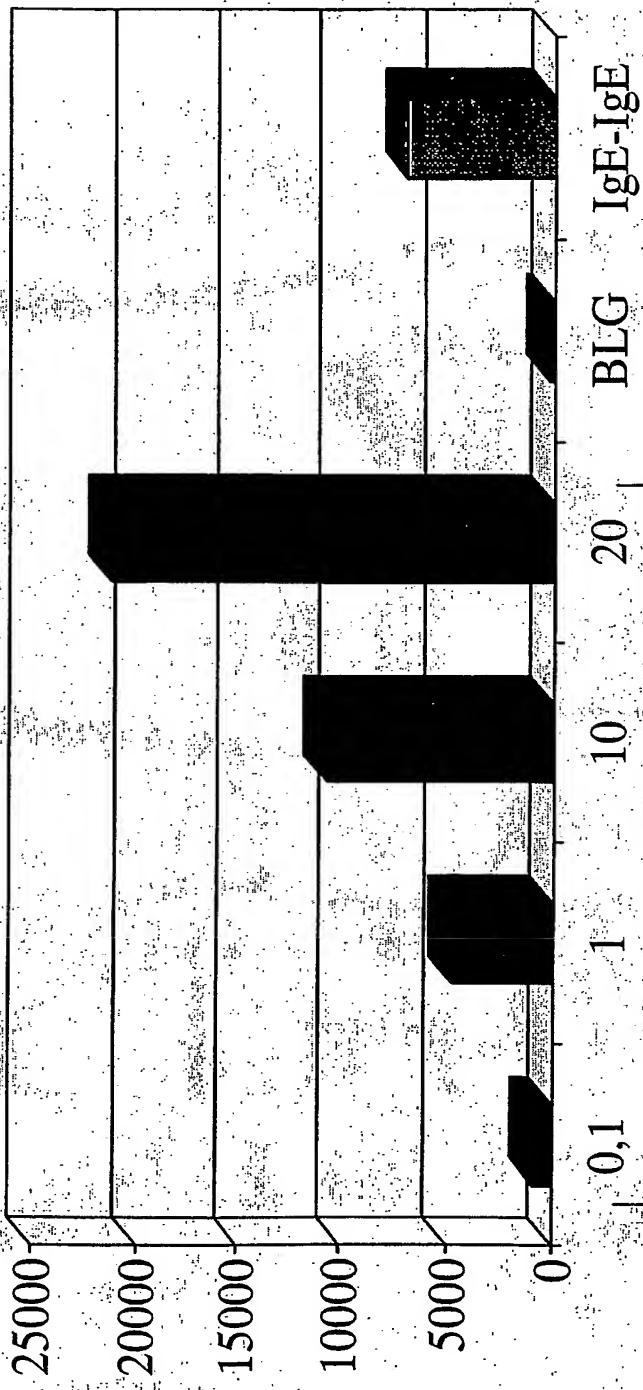


Figure 17

*In vitro stimulation of whole blood :  
dose-response to Feld1*

*IL-4 mRNA copy numbers per million of  $\beta$  actin mRNA*

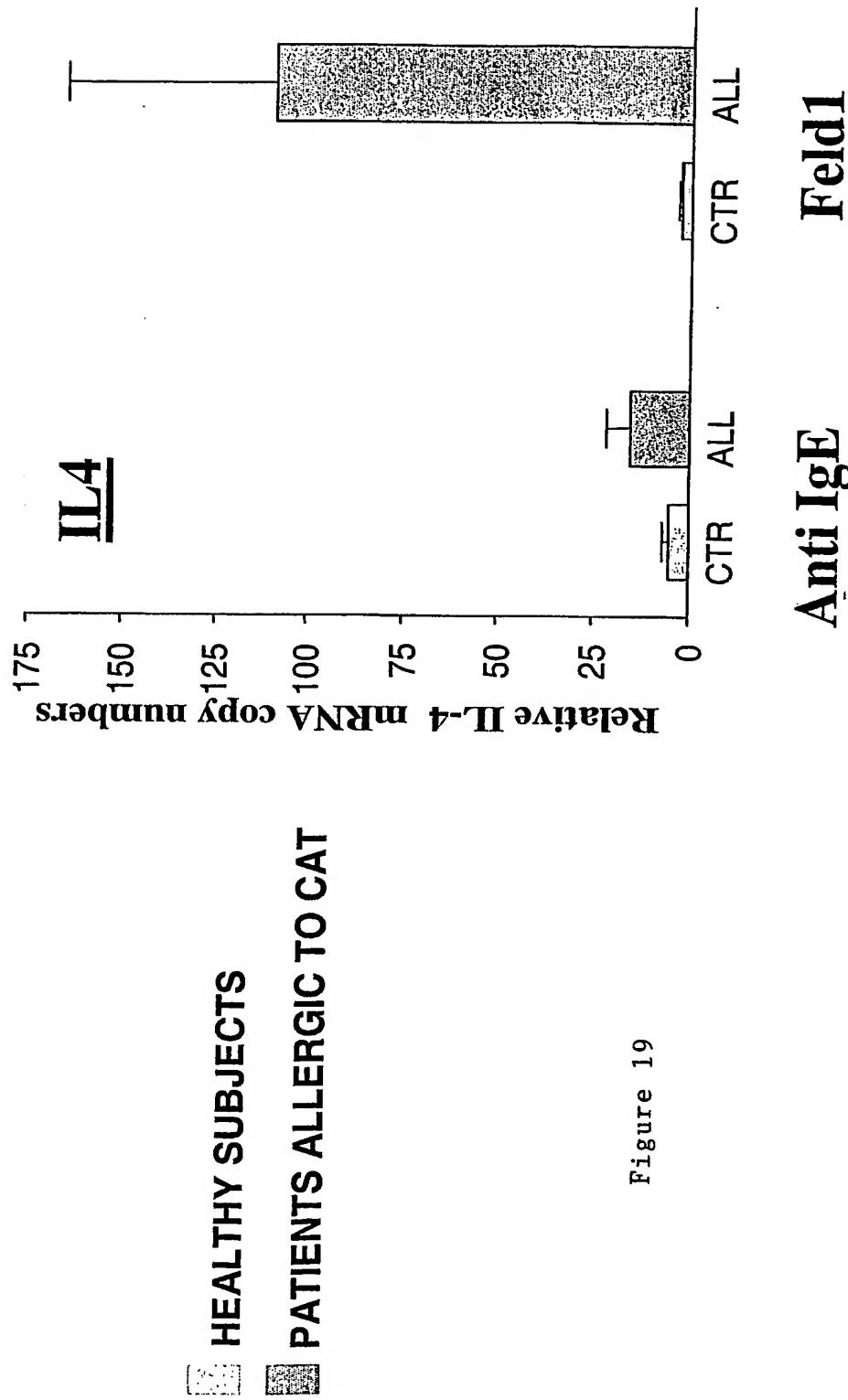


$\mu$ g/ml Feld1 allergen  
(2 hours of culture)

Figure 18

10/501666

**IL-4 mRNA levels after whole blood stimulation with Feld1 are higher in allergic patients compared to healthy controls**



# *In vitro* stimulation of whole blood : assessment of T cell response to GAD65

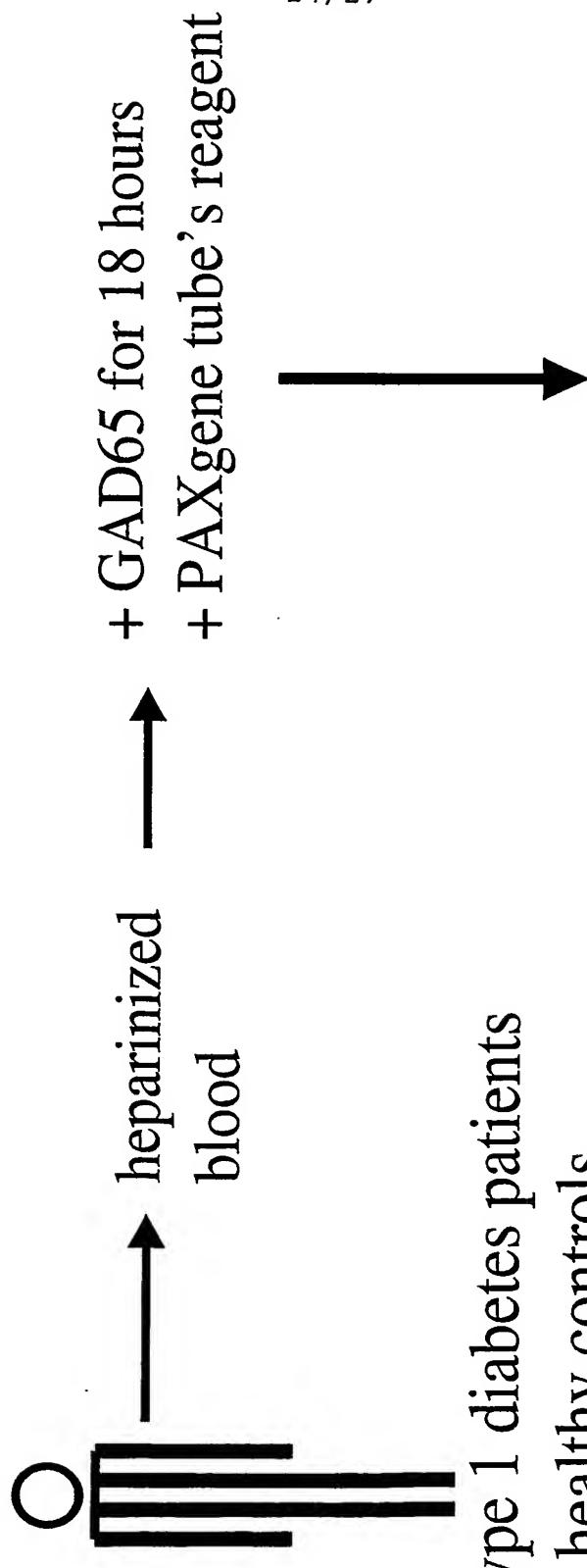


Figure 20

*In vitro* stimulation of whole blood:  
assessment of T cell response to GAD65

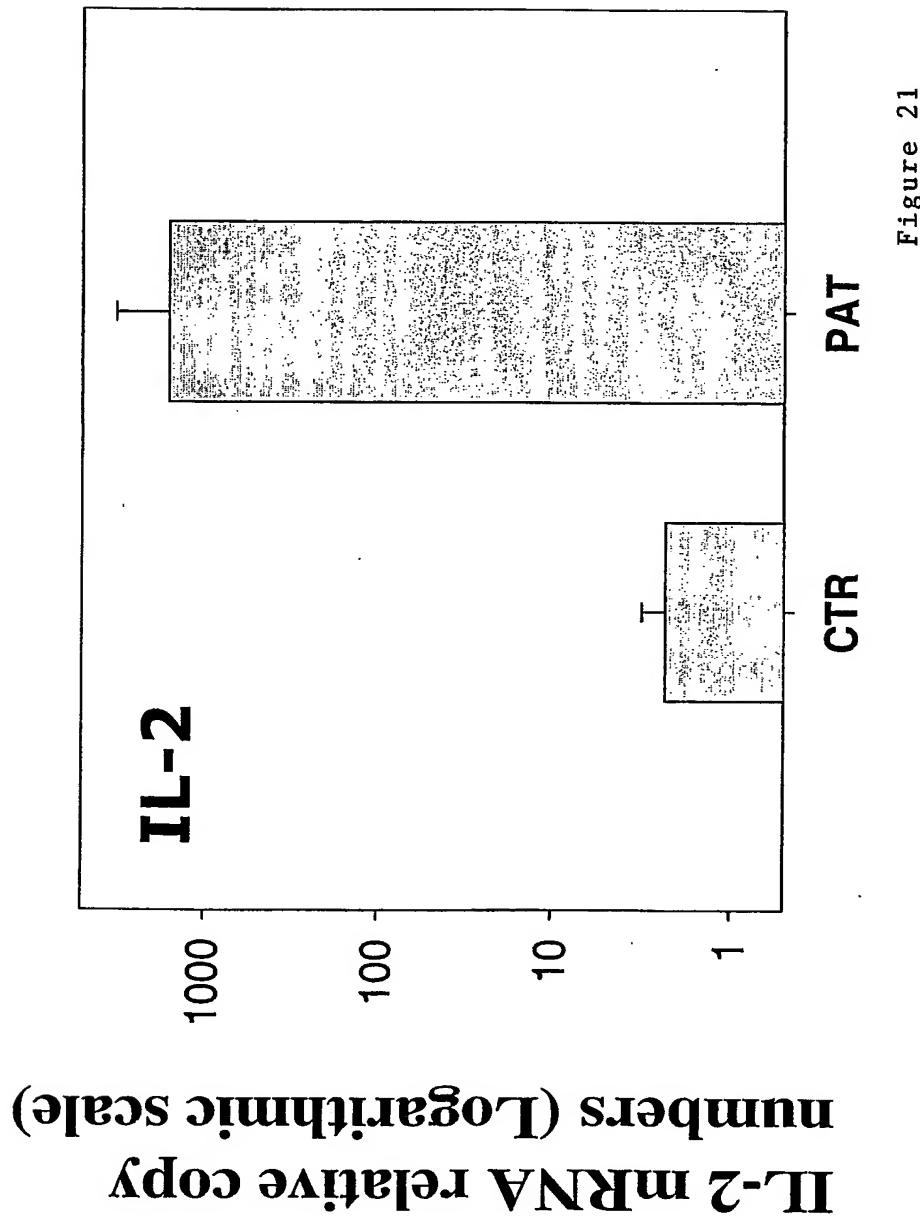


Figure 21

**Monitoring of alloreactive immune response :  
quantification of IL-2 mRNA in a whole blood +  
dendritic cells system**

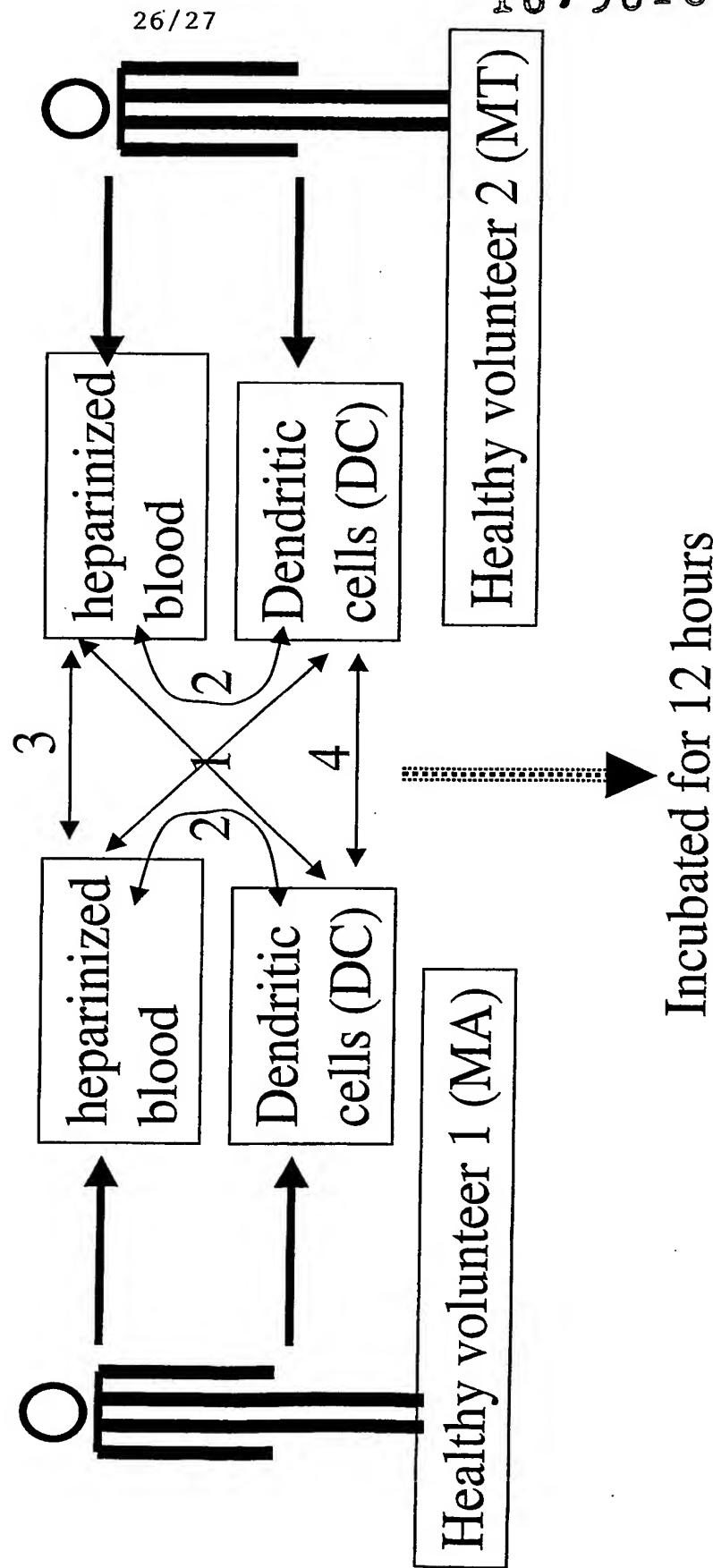
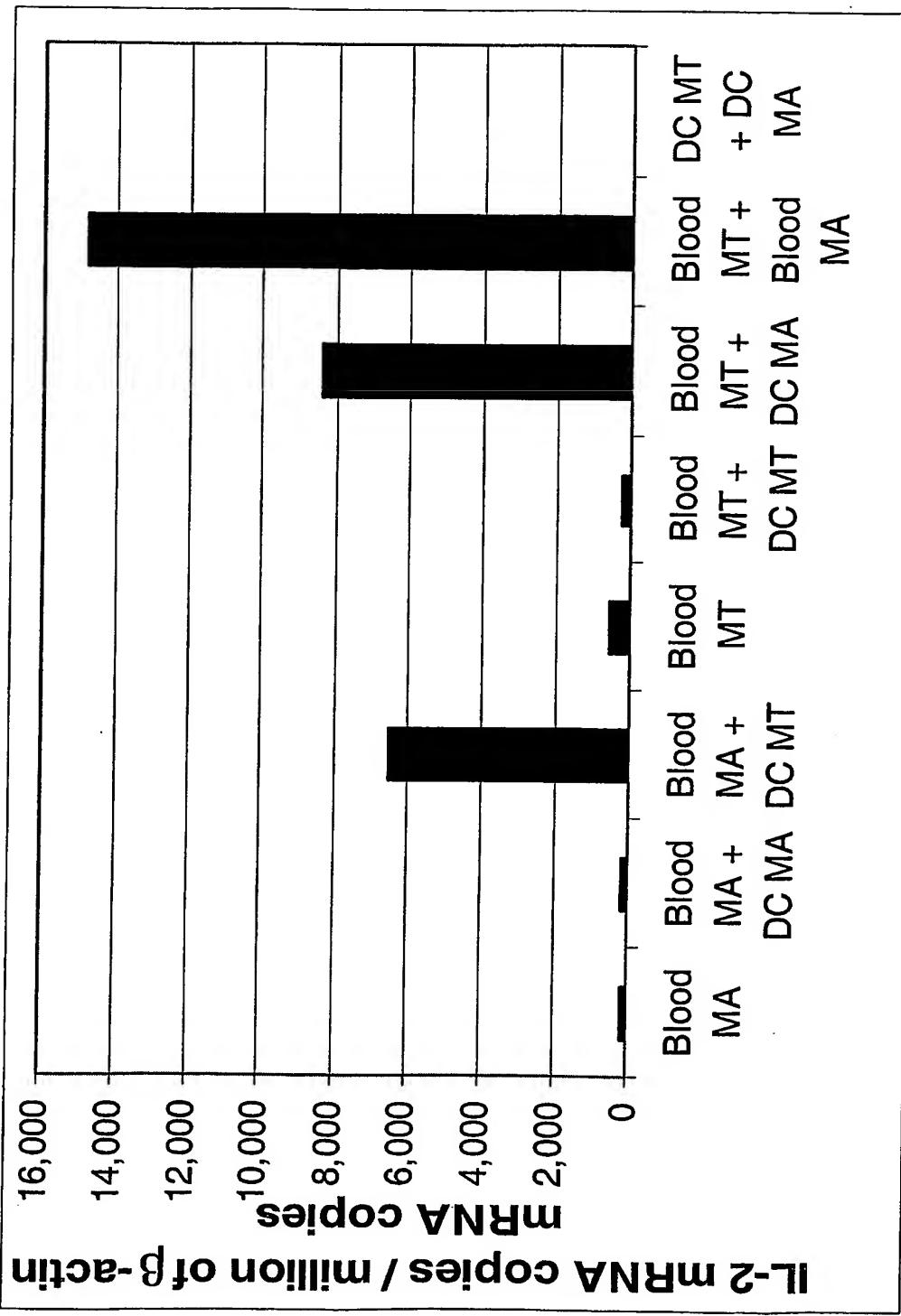


Figure 22

Monitoring of alloreactive immune response :  
quantification of IL-2 mRNA in a whole blood +  
dendritic cells system

Figure 23



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